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[54] CAPILLARY ELECTROPHORESIS ENZYME IMMUNOASSAY

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Related U.S. Application Data

[63] Continuation-in-part of application No. 08/386,224, Feb. 9, 1995, Pat. No. 5,810,985, which is a continuation of application No. 07/944,846, Sep. 14, 1992, abandoned.

[51] **Int. Cl.**⁶ **G01N 27/26**; G01N 33/53; G01N 33/561; C12Q 1/68

[52] **U.S. Cl.** **204/451**; 204/601; 435/6; 435/7.7; 435/7.92; 436/516

435/4, 7.7, 6, 7.91, 7.92, 7.9; 436/515, 516

[56] References Cited

U.S. PATENT DOCUMENTS

| 4,181,589 | 1/1980 | Brooks 4 | 35/173.9 |
|-----------|--------|-----------|----------|
| 4,288,425 | 9/1981 | Lee et al | 436/516 |
| 4,312,727 | 1/1982 | Shainoff | 204/469 |

(List continued on next page.)

FOREIGN PATENT DOCUMENTS

2 040 3132 8/1980 United Kingdom . WO 91/06850 5/1991 WIPO . WO 94/07132 3/1994 WIPO . WO 95/20161 7/1995 WIPO . WO 97/22825 6/1997 WIPO .

OTHER PUBLICATIONS

Schmalzing et al. (1995), "Solution—Phase Immunoassay for Determination of Cortisol in Serum by Capillary Electrophoresis," 41 *Clin. Chem.* 9:1403–1406, month unknown. Caplus abstract of Masanari Imamura ("A new quantitative determination of antigen by capilary agarose immunoelectrophoresis", Toho Igakkai Zasshi, 1977, 24(4), 612–15), month unavailable.

Masanari Imamura ("A new quantitative detrmination of antigen by capillary agarose imunoelectrophoresis", Toho Igakkai Zasshi, 1977, 24(4), 612–15), month uavailable. Aebersold et al., "Analysis of Dilute Peptide Samples by Capillary Zone Electrophoresis," *J. Chromatogr.*, 516:79–88 (1990), month unavailable.

Chien et al., "Field Amplified Sample Injection in High-Performance Capillary Electrophoresis," *J. Chromatogr.*, 559:141–152 (1991), month unavailable.

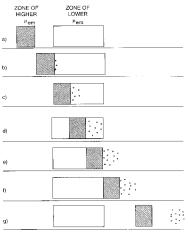
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[57] ABSTRACT

Disclosed are methods and apparatus for performing highly sensitive enzyme-amplified assays using channel electrophoresis of an enzyme having a biorecognition moiety. Combining a binding reaction of an analyte and an enzyme having a biorecognition moiety, and an electrophoretic separation which uses enzyme-amplified detection methodology, assay methods and apparatus are provided that offer simplicity, quantitative sensitivity and rapid analysis. Also disclosed are highly sensitive catalytically-amplified assays using a capillary electrophoresis format in which the binding reaction prior to electrophoresis is heterogeneous or homogeneous, and direct or competitive. The methods and apparatus for conducting sensitive automated catalytically-amplified assays using electrophoresis may be conducted in a microscale format.

29 Claims, 23 Drawing Sheets



RELATIVE POSITION ALONG CAPILLARY